

WHAT IS CLAIMED IS:

1. A method of producing a fucosylated carbohydrate in a single reaction mixture comprising the steps of:

5 (a) using a fucosyltransferase to form an O-glycosidic bond between a nucleoside 5'-diphosphofucose and an available hydroxyl group of a carbohydrate acceptor molecule to yield a fucosylated carbohydrate and a nucleoside 5'-diphosphate, and;

10 (b) recycling in situ the nucleoside 5'-diphosphate with fucose to form the corresponding nucleoside 5'-diphosphofucose.

15 2. The method of claim 1 wherein the base of the nucleoside is guanine.

20 3. The method of claim 1 wherein the nucleoside 5'-diphosphate is present in a catalytic amount.

25 4. The method of claim 1 wherein the hydroxyl group of the carbohydrate acceptor molecule is a part of an N-acetylglucosamine, galactose or N-acetylgalactosamine.

5. The method of claim 1 wherein the carbohydrate acceptor molecule is sialylated.

30 6. The method of claim 1 for producing a fucosylated sialylated carbohydrate molecule through enzymatic formation of glycosidic linkages in a single reaction mixture comprising:

00902600
" 0092600
44074

(a) forming a first glycosidic linkage between an diphosphonucleoside-activated glycosyl donor and an available hydroxyl group of a carbohydrate acceptor molecule using a first glycosyltransferase;

5 (b) forming a second glycosidic linkage between a monophosphonucleoside-activated sialyl donor and an available hydroxyl group of the carbohydrate acceptor molecule using a sialyltransferase; and

10 (c) forming a third glycosidic linkage between a diphosphonucleoside-activated fucosyl donor and an available hydroxyl group of the carbohydrate acceptor molecule by the method of claim 1, said glycosidic linkages of steps (a), (b) and (c) being formed in a single reaction mixture.

15 7. The method of claim 6 wherein the fucosylated sialylated carbohydrate moiety is a sialylated Lewis ligand.

20 8. The method of claim 7 wherein the sialylated Lewis ligand is SLe^x or SLe^a .

25 9. The method of claim 6 wherein the fucose is transferred from a fucosyl donor to a hydroxyl group of a N-acetylglucosamine residue of the carbohydrate acceptor molecule.

30 10. The method of claim 9 wherein the fucosyl donor transfers fucose to a hydroxyl group of carbon 3 of the N-acetylglucosamine.

35 11. The method of claim 6 wherein the fucosyl donor transfers fucose to a hydroxyl group of a galactose residue of the carbohydrate acceptor molecule.

12. The method of claim 6 wherein the sialyltransferase is selected from the group consisting of α 2,3 sialyltransferase, α 2,4 sialyltransferase, α 2,6 sialyltransferase and α 2,8 sialyltransferase.

13. The method of claim 6 wherein the fucosyltransferase is selected from the group consisting of α 1,2 fucosyltransferase, α 1,3 fucosyltransferase, α 1,6 fucosyltransferase, α 1,4 fucosyltransferase and α 1,3/4 fucosyltransferase.

14. The method of claim 6 wherein the fucosyltransferase is selected from the group consisting of β -galactosidase α 1,2 fucosyltransferase, N-acetylglucosamine α 1,3 fucosyltransferase, N-acetylglucosamine α 1,4 fucosyltransferase, N-acetylglucosamine α 1,6 fucosyltransferase and N-acetylglucosamine α 1,3/4 fucosyltransferase.

15. The method of claim 6 wherein the carbohydrate acceptor molecule is a carbohydrate substituted molecule wherein the carbohydrate terminates in a Gal β 1,4GlcNAc-X where X is an organic molecule.

16. The method of claim 6 wherein the base of at least one nucleoside is either cytidine or uridine.

17. The method of claim 6 wherein the monophosphonucleoside-activated sialyl donor is cytidine 5'-monophospho-N-acetylneuraminic acid.

18. The method of claim 6 wherein the diphosphonucleoside-activated fucosyl donor is guanosine 5'-diphospho-fucose.

19. The method of claim 6 wherein the glycosyl donor of step (a) is a diphosphonucleoside-activated galactosyl donor that is uridine 5'-diphospho-galactose.

5

20. The method of claim 6 wherein steps (a) and (b) are substantially completed before step (c) is begun.

10

21. An in vitro reaction system comprising a fucosyltransferase and a nucleoside-diphospho fucose forming enzyme.

sp. which compounds are in this?

15

22. The reaction system of claim 21 wherein the nucleoside-diphospho fucose-forming enzyme is guanosine diphospho-fucose pyrophosphorylase.

20

23. The reaction system of claim 21 which further comprises a kinase.

25

24. The in vitro reaction system of claim 23 further comprising a pyruvate kinase.

antecedent basis

25. The reaction system of claim ^{23?} 24 wherein the kinase is a fucose kinase.

26. The reaction system of claim 22 further comprising a NADPH regeneration system.

Sub A2

30

27. The reaction-system of claim 26 wherein the nucleoside diphospho fucose forming system contains guanosine diphospho-mannose.

antecedent basis?

28. The system of ^{in hand} claim 27 wherein guanosine diphosphate mannose is generated in situ from guanosine triphosphate and mannose 1-phosphate.

5 29. The system of claim 28 which further comprises pyruvate kinase and guanosine diphospho-mannose pyrophosphorylase.

10 30. A method for production of a glycosyl 1- or 2-phosphite which comprises reacting a blocked glycosyl ring having a hydroxyl at the anomeric position with a trivalent phosphitylating reagent to yield a blocked glycosyl 1- or 2-phosphite-substituted ring.

15 31. The method of claim 30 wherein the glycosyl ring is selected from the group consisting of galactosyl, glucosyl, fucosyl, N-acetylglucosaminyl, mannosyl and 8- or 9-carbon sugars having a carboxyl or C₁-C₅ alkyl or benzyl carboxylate ester at the
20 1-position.

32. The method of claim 30 wherein the glycosyl ring is fucosyl.

25 33. The method of claim 31 wherein the trivalent phosphitylating reagent is a dibenzyl N,N-dialkylphosphoroamidite.

30 34. The method of claim 31 wherein the dibenzyl N,N-dialkylphosphoroamidite is dibenzyl N,N-diethylphosphoroamidite.

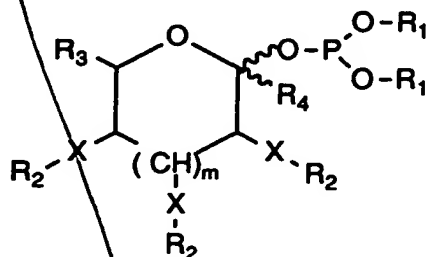
35 35. The method of claim 31 wherein the glycosyl ring is blocked with acetyl or benzyl blocking groups.

36. The method of claim 31 wherein the glycosyl ring is selected from the group consisting of L- or D-aldoses having four, five or six carbons.

5 37. The method of claim 31 wherein the glycosyl ring is selected from the group consisting of L- or D-ketoses having four, five or six carbons.

10 38. The method of claim 30 wherein the glycosyl ring is selected from the group consisting of D- and L-aldoses and ketoses having four through nine carbons.

15 39. The blocked phosphityl monosaccharide of the formula:



25 wherein each R_1 is the same or different and is an aryl group or a C_1 - C_5 lower alkyl group; X is independently oxygen or nitrogen; R_2 is independently an acyl, benzyl, silyl or alkyl blocking group or X - R_2 together are absent and are replaced by hydrogen;

30 R_3 is independently hydrogen, $-CH_3$, $-OR_2$, $-CH_2OR_2$, $-CH(OR_2)-CH(OR_2)$, or $-CH(OR_2)-CH(OR_2)-CH(OR_2)$;

R_4 is hydrogen, carboxyl or C_1 - C_5 alkyl or benzyl carboxylate; and

m is zero or 1.

35

40. The compound of claim 39 wherein m is 1 and each X is oxygen.

5 41. The compound of claim 39 wherein the monosaccharide is selected from the group consisting of mannose, fucose, galactose, glucose and rhamnose.

10 42. The compound of claim 39 wherein m is 1, one X is nitrogen and the other X's are oxygen.

15 43. The compound of claim 42 wherein the monosaccharide is selected from the group consisting of 2-acetamido-2-deoxy-galactose, 2-acetamido-2-deoxy-glucose, N-acetyl-neuraminic acid and 2-phalimidoyl-2-deoxy-glucose.

44. The compound of claim 39 wherein R₁ is benzyl.

20 45. The compound of claim 39 wherein R₂ is benzyl or acetyl.

25 46. The compound of claim 39 that is selected from the group consisting of

dibenzylphosphinyl 2,3,4,6-tetra-O-acetyl- α -D-mannoside,
dibenzylphosphinyl 2,3,4,-tri-O-acetyl- β -L-fucoside,

30 dibenzylphosphinyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-D-galactoside,
dibenzylphosphinyl 2-acetamido-2-deoxy-3,4,6-tri-O-acetyl-D-glucose,
dibenzylphosphinyl 2,3,4,6-tetra-O-acetyl-D-galactoside,

dibenzylphosphinyl 2,3,4,6-tetra-O-acetyl-D-glucoside,
dibenzylphosphinyl 2,3,4-tri-O-acetyl-L-rhamnoside,
5 dibenzylphosphinyl 2-phthalimidoyl-2-deoxy-3,4,6-tri-O-acetyl-D-glucoside, and
methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-2-(dibenzylphosphityl)-3,5-dideoxy- β -D-glycero-D-galacto-2-nonulopyranosonate.

10

47. A compound that is 2,3,4-tri-O-benzoyl- α -L-fucopyranosyl bromide.

15

48. A process of halohydration that comprises
(a) admixing a glycal with hydrogen peroxide, a halide ion selected from the group consisting of chloride, bromide and iodide and a catalytic amount of a chloroperoxidase enzyme in an aqueous buffer having a pH value of about 2.5 to about
20 3.5 to form a reaction mixture; and

(b) maintaining the reaction mixture so formed for a time period sufficient for halohydration to proceed and a halohydrin product to form.

25

49. The process of claim 48 including the further step of recovering the halohydrin product.

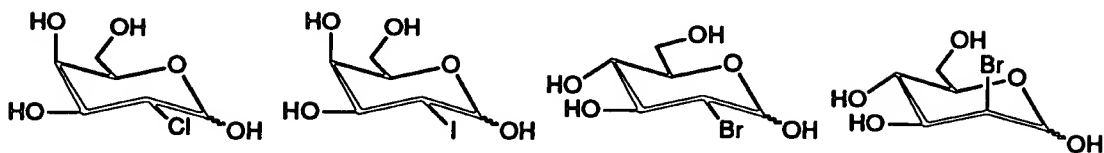
30

50. The product of the process of claim 48.

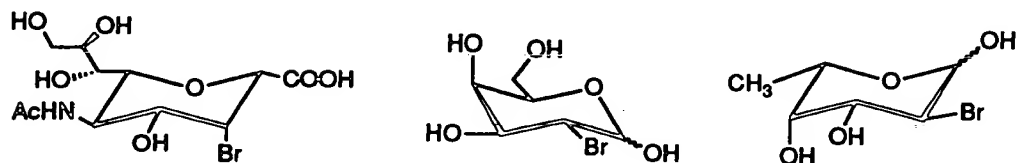
51. A glycosylhalohydrin having a formula selected from the group consisting of

III.

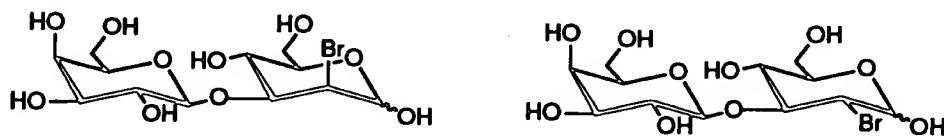
Species
which
one?



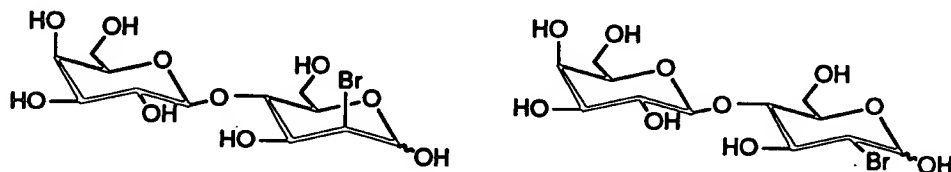
10



15

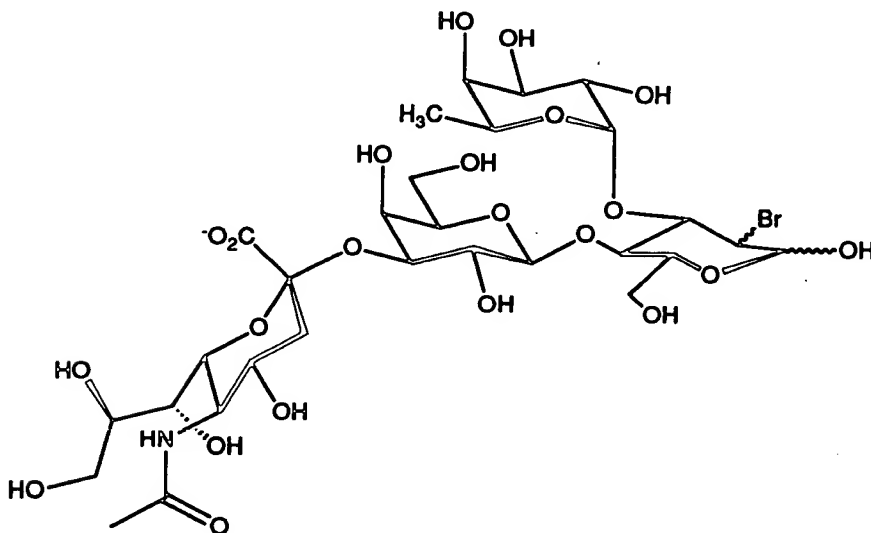


20



25

30



35

FIG. 10